

PTL 73288

# **MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD- MAPPING**

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**Seventh Progress Report  
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Neural Prosthesis Program**

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## **I. Introduction**

Previous studies from this laboratory have used electrical stimulation delivered via single fine-tipped microelectrodes to map sites in the lumbosacral spinal cord which produce bladder contractions, penile erection, modulation of external urethral sphincter activity or flexion and extension of the hindlimb about the knee joint. During this quarter we began experiments using a fixed array of four microelectrodes to examine the possibility of improving the extension torque generated by microstimulation at several sites in the L<sub>6</sub> spinal cord. The stimuli were present either simultaneously to each electrode or by interleaving the stimuli in various patterns. The results indicate that with stimulation of more than one electrode the response (extension torque) can be greatly increased at the same time the current presented to each electrode can be reduced. The effects of interleaving of stimuli, distances between electrodes, number of electrodes activated, and changes in stimulus parameters are also examined and discussed in the report presented below.

During this quarter neural tracing with pseudorabies virus and single electrode mapping studies also continued during this quarter.

Since multiple electrode stimulation is an important consideration in designing future experiments for both somatomotor (hindlimb extension-flexion) and autonomic (bladder, penis, urethra, etc.) neural activation, the results from these experiments will be described in detail in this progress report.

## **II. Hindlimb Flexion and Extension Elicited by Microstimulation of the Spinal Cord With Four Microelectrodes**

### *A. Methods*

The methods used in these studies have been described in previous progress reports and are summarized below. New techniques for dealing with four stimulating electrodes and data recording and analysis are described in detail.

Male cats weighing from 3.5 to 5.0 kg were used in these studies. The animals were anesthetized with halothane:oxygen during surgery and with pentobarbital (25mg/kg iv and supplemented as needed) during the experiments. Extension and flexion isometric torque was

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recorded from the left hindlimb by a rotational torque transducer attached to the shank (lower hindlimb) with the pivot point centered at the knee joint. Electromyographs (EMGs) were recorded from fine wire electrodes placed in the shank extensor (quadriceps) and flexor (biceps femoris) muscles. Since the torque sensor recorded net torque generated at the shank by the flexors and extensors, the EMG recordings provided important information concerning the selectivity of muscle activation. With microelectrode stimulation of the L<sub>6</sub> spinal cord the stimulus parameters and electrode position could be adjusted to produce extensor torque with high amplitude EMG activity in the extensor muscles and little or no EMG activity in the flexors. The selectivity for extensor muscles was somewhat easier to obtain in the L<sub>6</sub> spinal cord since many of the motoneurons in L<sub>6</sub> innervate shank extensor muscles. Stimulation of the L<sub>6</sub> ventral root which activates the entire L<sub>6</sub> motor output produces a net torque response but the EMG shows both extensor and flexor activity with the extensor EMG having a larger amplitude. Spinal cord microstimulation at specific sites can produce a more selective activation of the extensors and these responses are reflected in the relative amplitude of the EMG activity (Fig. 1).

Four microelectrodes were used in the present experiment to stimulate neurons in the spinal cord. The electrodes used were standard activated iridium electrodes used for stimulus mapping of the spinal cord. The exposed surface area of each electrode was 300 sq. microns. The electrodes were mounted in a holder at a fixed position, with the tip at the same level and separated by a center-to-center distance of 500 $\mu$ . Since individual electrodes could not be moved independently the electrode array was lowered into the spinal cord as a single unit. The array was lowered at 200 $\mu$  increments and the spinal cord stimulated with each electrode singly and in various combinations. The array was oriented in a rostrocaudal direction with electrode A being the most rostral and D the most caudal (see Fig. 1, bottom). The rostrocaudal orientation approximated the parallel orientation of the motor cell columns which innervate specific muscle groups.

The stimuli were applied to two or more electrodes either simultaneously or interleaved at specific delays between presentation of the stimuli to each electrode. The stimulus parameters were 40 Hz, 0.2 msec pulse duration, at 10 - 100  $\mu$ A for 10 to 30 sec on and 120 sec off. The interleave delay at 40 Hz, with two electrodes varied from 0.5 to 12.5 msec and for three

electrodes from 0.5 to 8 msec.

With 30 sec stimulus duration the torque generated by the muscle first increases with time then slowly decreases. The decrease in muscle torque is termed fatigue. Fatigue in our experiment is defined as the difference between the maximal torque produced during the 30 sec stimulus and the torque just before the end of stimulation. The position of the electrode within the spinal cord was determined histologically at the end of the experiment.

Throughout all of these experiments there were a number of important considerations based on previous experiments using single microelectrodes, and a number of questions that were trying to be answered. These include: 1) What is the best spacing between electrodes to maximize the response? 2) What is the best orientation for the electrode array? Rostrocaudal or medial-lateral? 3) What types of interactions occur when two or more electrodes are used to produce a stimulus generated response? Are they additive, synergistic, inhibitory or produce no effect? 4) Our previous studies with single microelectrodes indicate that muscle fatigue can be controlled by optimizing stimulus parameters, especially frequency and intensity of stimulation. Can fatigue be reduced further with multiple electrode stimulation and are the same stimulus parameters important?

### *B. Results*

These results are based on two animals in which 32 electrode tracks were identified histologically and correlated with the torque responses and EMGs from the hindlimb. Figure 1 shows typical torque and EMG responses from each of the four electrodes in the array. The electrodes are separated by a distance of  $500\mu$  and the recordings are made from a depth of 4.2 mm from the  $L_6$  spinal cord surface (Fig. 1, bottom). Stimulation of each electrode produces a slightly different response, although adjacent electrodes often have almost identical responses, suggesting that at  $500\mu$ , separation of electrodes may stimulate nearly the same group of neurons and processes. The EMG recordings in Figure 1 would suggest the selectivity of each electrode for extension is very good. The small baseline activity seen in the flexor EMG recording in Figure 1 is stimulus artifact being recorded by the EMG electrodes.

The torque response profile had three phases that can be identified (Fig. 1): an initial

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rapid rise in torque when stimulation is initiated, followed by a slower increase in torque during the next 5 - 10 seconds, and finally a slow decrease in torque over the last 15 - 20 seconds, and a rapid return to baseline when the stimulation ends. The fatigue which occurs with a 30 sec stimulus using a single electrode is minimal at 40 Hz; the frequency used in present experiments.

Further evidence that adjacent electrodes with a  $500\mu$  separation may stimulate a similar or overlapping group of neurons is seen in Figure 2, where the maximal torque is plotted for each  $200\mu$  increment in depth along each of the four electrode tracks. Electrodes A and B; and C and D produce similar responses, however it should also be noted that electrodes B and C, which are also adjacent, have quite different responses. The important question however, is not whether adjacent electrodes stimulate the same or similar groups of neurons (at higher intensities of simulation they probably do) but whether two or more electrodes are better than one in enhancing the response and is there an optimal degree of separation?

It is clear that two or more electrodes can produce an enhanced response compared to a single electrode. The optimal distance between electrodes is less clear although at least additive effects are seen at 500 -  $1500\mu$  separation, with some synergistic (greater than additive) effect elicited by electrode separation of 500 -  $1000\mu$ . Distances greater than  $1500\mu$  have not yet been examined.

The additive and synergistic effects of two or more electrodes are illustrated in Figures 3 and 7. These figures show that microstimulation with two electrodes are better than one, and three are better than two in enhancing the extension torque to  $L_6$  cord stimulation. Furthermore, simulation with two or three adjacent electrodes produces at least an additive effect and in many instances a greater than additive or synergistic response. The synergism is most dramatic at low intensities of simulation and with three electrodes activated simultaneously (Figs. 3 and 7). For example, at  $40\mu A$  (Fig. 3 best seen in Fig. 7) individual electrodes B and C are just at or below threshold for an extension response while electrode D produces a small response. However, when B and C; or B and D; or C and D are stimulated together, the response is greater than the algebraic sum of the individual responses. This is an important technique which should allow the reduction in current density at each electrode while still enhancing the end organ responses.

When using more than one stimulating electrode to activate a group of neurons, a

technique used to enhance responses is to present the stimuli in an interleaved mode rather than simultaneously. If using two electrodes for example, a stimulus would be delivered to the tissue via electrode A, followed by some delay and a stimulus would be delivered by electrode B. The cycle would then be repeated a set number of times. When using three or more electrodes all electrodes could be interleaved or a combination of interleaving and simultaneous stimulation could be used. We used interleaving stimuli in our experiments with two and three electrodes to determine if an enhancement in torque response would occur and also to see if a reduction in fatigue of the extensor torque could be produced by interleaving (Figs. 4, 5, & 6). Also examined was the relation of stimulus interleaving with intensity of stimulation (Figs. 5 & 6). Interleaving of stimuli proved to be a disappointment. Interleaving at various delays produced only a decrease in hindlimb torque (Fig. 4). This was seen at both high and low intensities of stimulation (Figs. 5 & 6). Furthermore, the reduction in fatigue was always accompanied by an equal reduction in response amplitude (Figs. 4, 5, & 6). Figure 4 indicates that only a small delay (0.5 msec) produced a dramatic drop in hindlimb torque. The decrease in response was elicited at all intensities of stimulation from 20  $\mu$ A to 100  $\mu$ A (Fig. 5). Similar reduction in torque was produced with two electrodes being interleaved (Fig. 6). Simply reducing the intensity of stimulation could produce the same effect as interleaving (Fig. 6).

The conclusions from these experiments are: 1) The use of multiple electrodes is a useful and important technique to enhance motor responses while reducing current density at a given electrode. 2) Preliminary studies with stimulus interleaving indicates that no advantage is produced over simultaneous stimulation with electrodes. Some advantage may be apparent with more than three electrodes, with various stimulus frequencies (only 40 Hz tried in these experiments) or with large spacing between electrodes. 3) The optimal spacing of electrodes and the best orientation (rostrocaudal, mediolateral, or expansion in both directions with more electrodes) is unclear at this time.

These types of studies will continue in the next quarter. Penile, bladder, and motor response will be examined with an array of four or more electrodes.

- Figure 1** Plots showing changes in flexion and extension torque (top of each panel) and EMG activity (middle and bottom of each panel) following stimulation of the L<sub>6</sub> spinal cord for each of four electrodes, labeled A, B, C, and D. Figure at bottom shows the location of each electrode in the L<sub>6</sub> spinal cord. Electrode A is most rostral and D most caudal. The inter-electrode distance is 500 $\mu$ . Time is in seconds. Stimulus was applied for 30 seconds beginning at 5 seconds and ending at 35 seconds. Stimulus parameters were 0.2 msec pulse duration, 40 Hz, 100  $\mu$ A, 30 sec. on 120 sec. off. The depth from spinal cord surface is 4.2 mm. Notice the correlation of extensor torque with the extensor EMG. The small flexor EMG trace is stimulus artifact picked up by the EMG recording electrodes.
- Figure 2** Graphs showing changes in maximal flexor and extensor torque at different depths from the surface of the L<sub>6</sub> spinal cord for four electrodes labeled A, B, C, and D. Stimulus parameters same as Figure 1. Also, see figure at bottom of Figure 1 for electrode separation and orientation. Notice the similarities between A and B; and C and D, these two electrodes may be activating the same neural population. B and C are also adjacent but quite different in their response profiles.
- Figure 3** Graph showing changes in maximal flexor and extensor torque to different stimulus intensities for each of three electrodes labeled A, B, and C and for combinations of two (B + C, B + D, and C + D) or three (B + C + D) electrodes used for L<sub>6</sub> cord stimulation. Stimuli were presented simultaneously when two or three electrodes were used. Stimulus parameters and orientation shown at bottom Figure 1. Notice that stimulation with a combination of two or three electrodes produced at least an additive effect and in most cases a greater than additive effect at most intensities of stimulation. See also Figure 7.
- Figure 4** Graph showing changes in response (hindlimb extension) and fatigue torque at various interleave delays. Three electrodes (B, C, and D) are used for microstimulation of the L<sub>6</sub> spinal cord. At 0 msec. interleave the stimuli are applied simultaneously to the spinal cord. Fatigue is the decrease in torque seen 30 seconds into the stimulus (see methods for details), while interleave time is the time delay for stimulation via the next electrode. Stimulus sequence was always from B to C to D. Stimulus parameters same as Figure 1. Electrodes were all 4.2 mm from surface of spinal cord. Notice the large decrease in torque (and also fatigue) with just a small delay in the stimulus to the second (C) and third (D) electrode.
- Figure 5** Graph showing changes in response (hindlimb extension) and fatigue torque at various intensities of microstimulation of the L<sub>6</sub> spinal cord via three electrodes (B, C, and D). Three interleave delays; are shown, 0, 1, and 6 msec. For definition of fatigue and interleave see Figure legend 4 and methods. Interleave delay of 0 ms is simultaneous stimulation via all three electrodes. Stimulus parameters given in

methods and Figure 1. Notice that at all intensities of stimulation that interleaved reduces the response. Fatigue is also reduced but at the expense of a large drop in response torque.

**Figure 6** Graph showing changes in response (hindlimb extension) and fatigue torque to changes in interleave delay for two electrodes A and B, at two intensities of stimulation,  $75 \mu\text{A}$  and  $100 \mu\text{A}$  (to both electrodes). Stimulus parameters same as Figure 1. Depth of both electrodes 4.0 mm. This track is lateral to that shown in Figure 1. Notice the decrease in response with interleave at both intensities. Also notice that fatigue can be reduced with interleave, but can also be reduced with a decrease in intensity of stimulation with out interleave.

**Figure 7** Bar graph showing changes in extension torque for single microelectrode simultaneous (B, C, and D) and various combinations of two (B + C, B + D, and C + D) and three (B + C + D) electrodes. Data from Figure 3 at  $40 \mu\text{A}$  and  $80 \mu\text{A}$  are replotted to show additive and synergistic effects of multiple electrode stimulation. Stimulus parameters and orientation same as Figure 1. Notice that stimulation with a single electrode B or C is below threshold at  $40 \mu\text{A}$  yet when used to stimulate the spinal cord together produced a measurable response. The use of three microelectrodes for stimulation produces a very large response. In almost every combination the response is greater than the additive response of single electrode stimulation.

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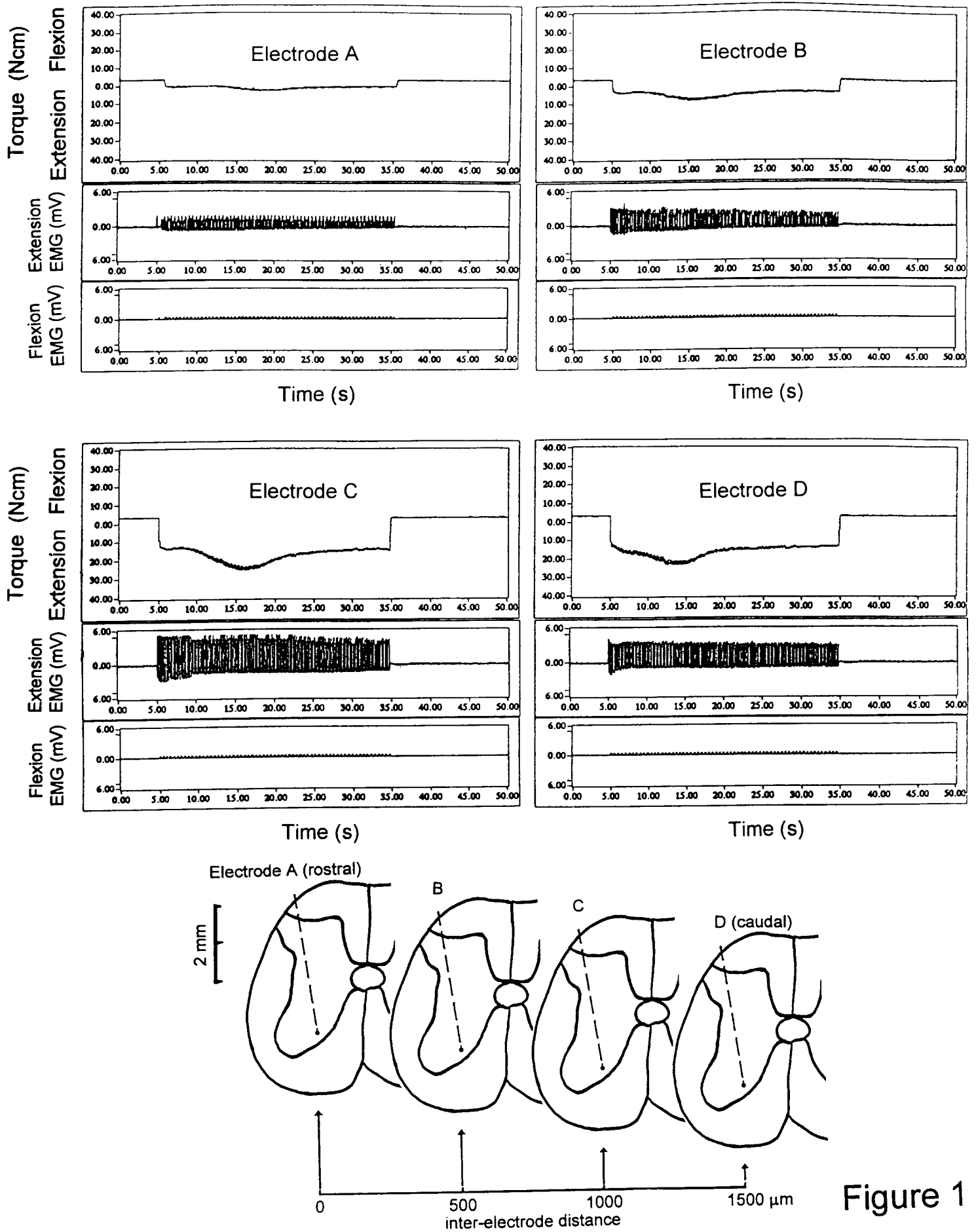


Figure 1

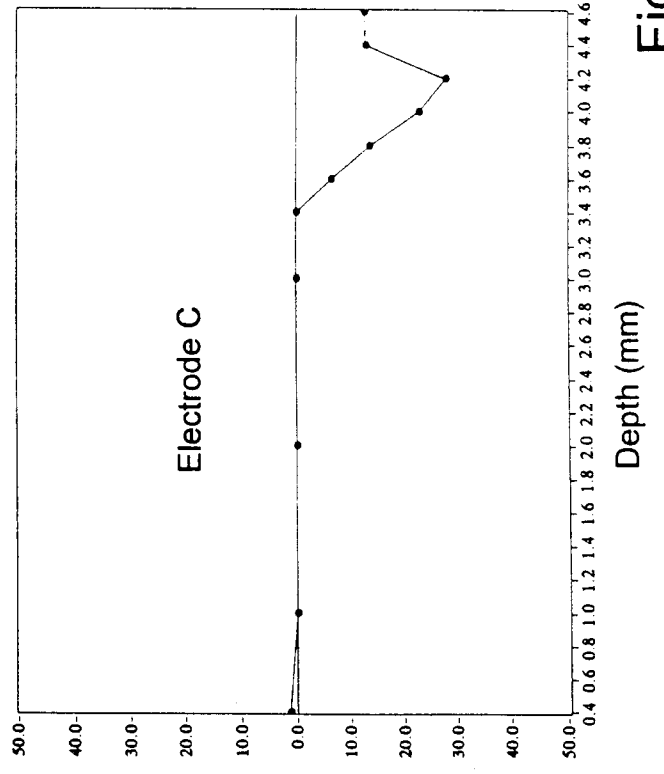
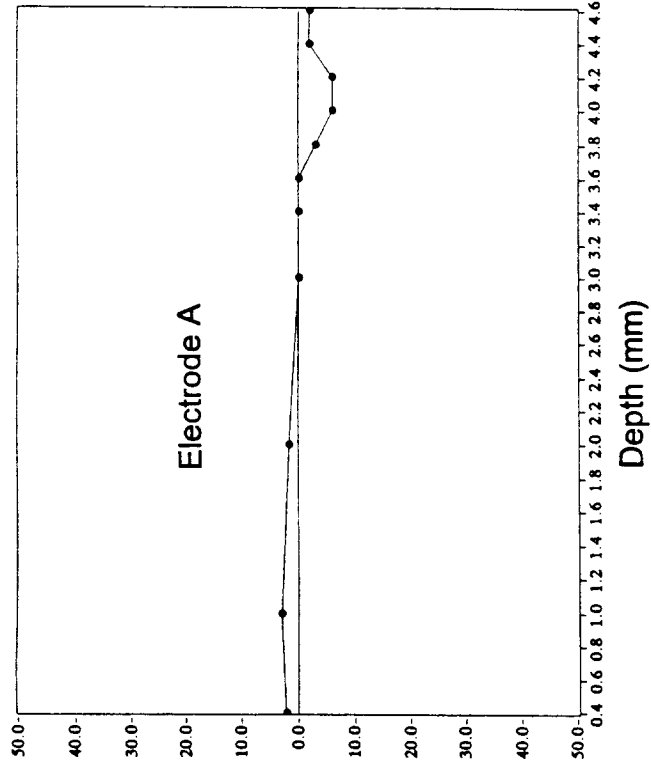
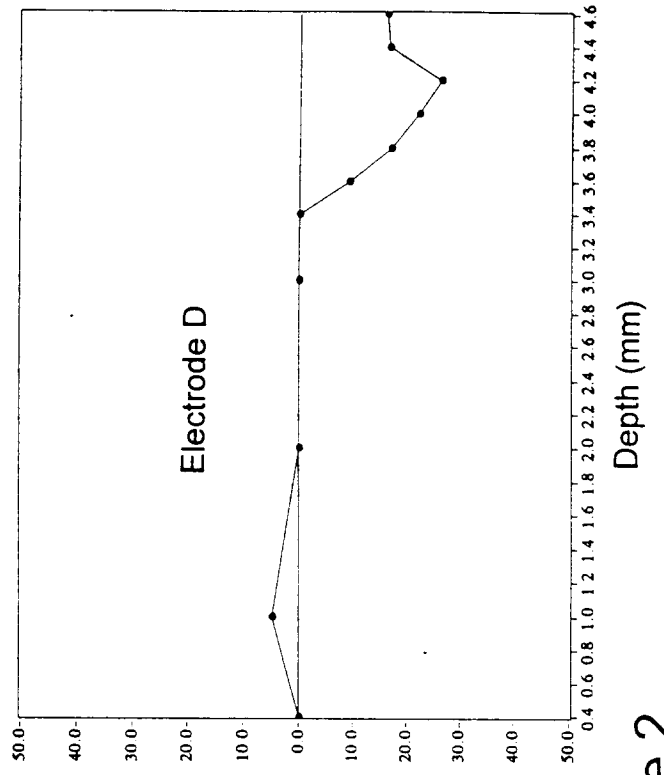
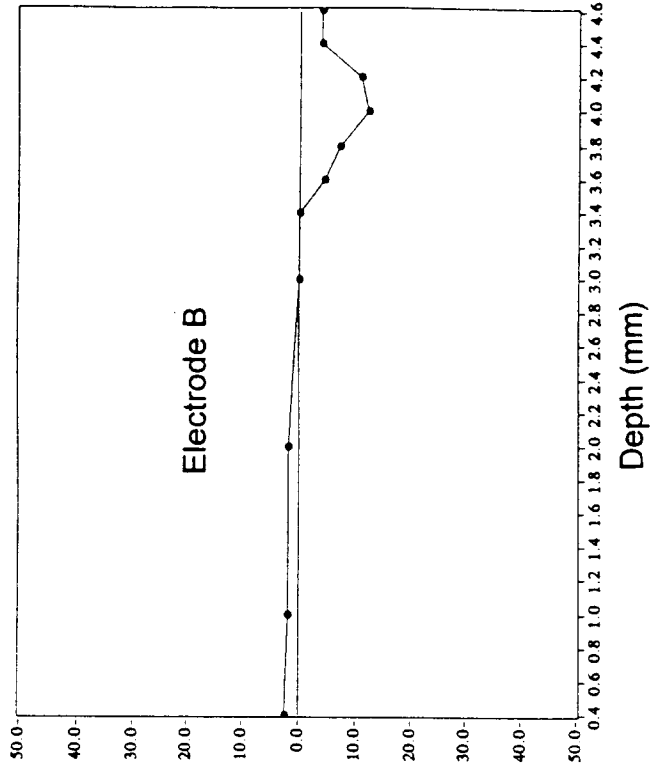


Figure 2

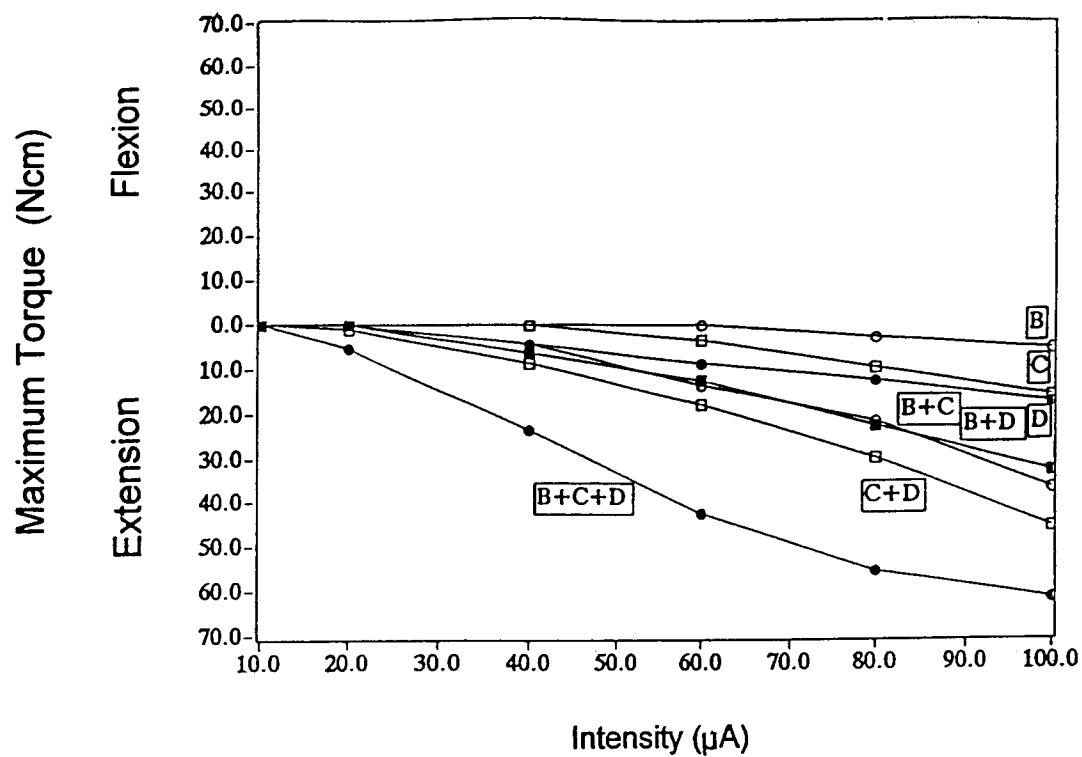


Figure 3

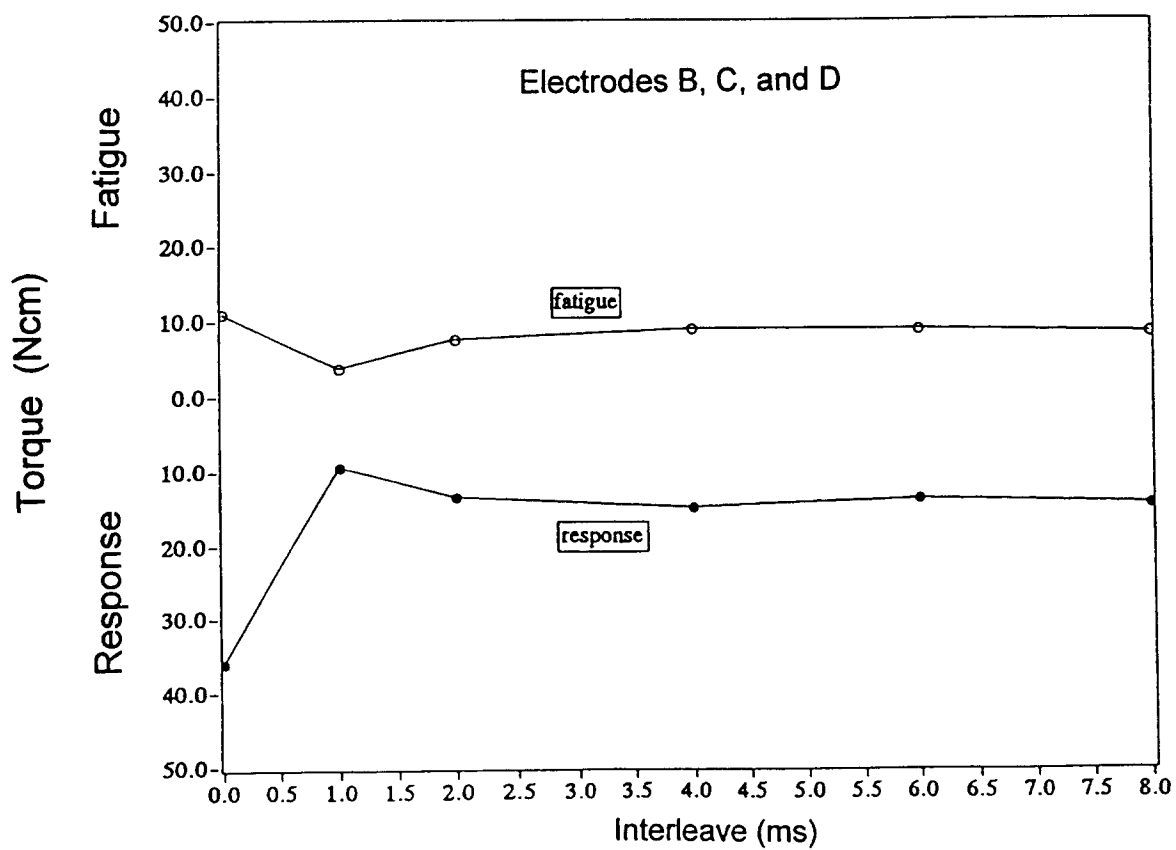


Figure 4

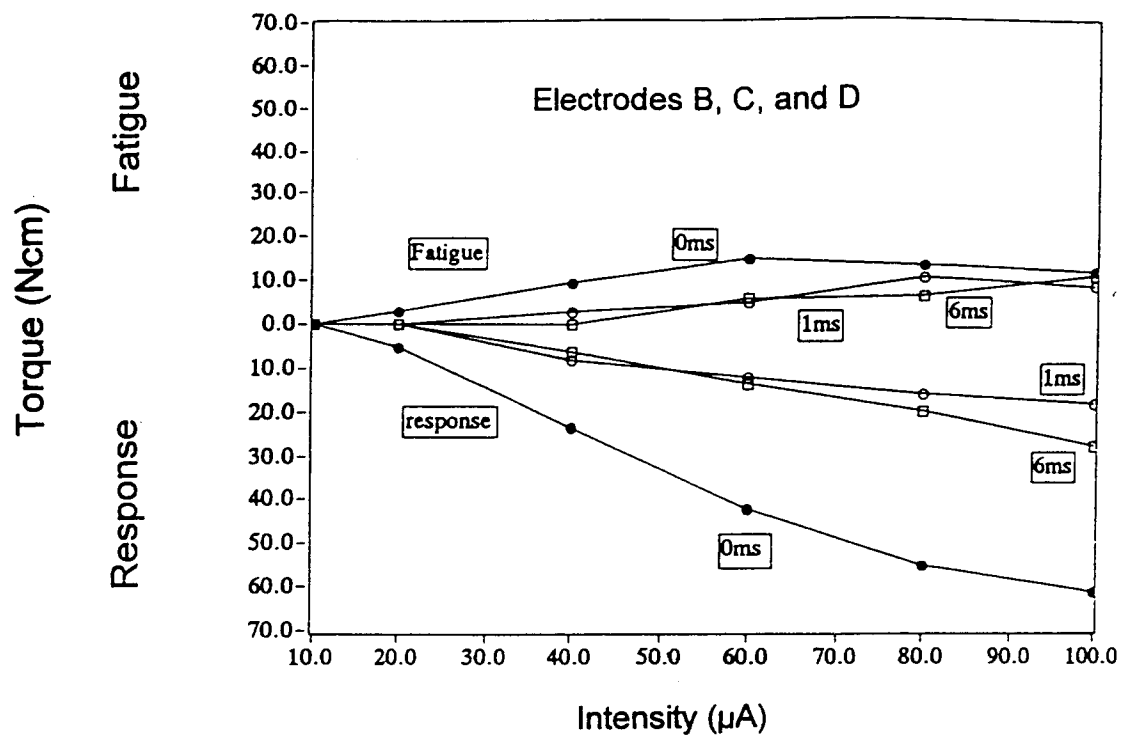


Figure 5

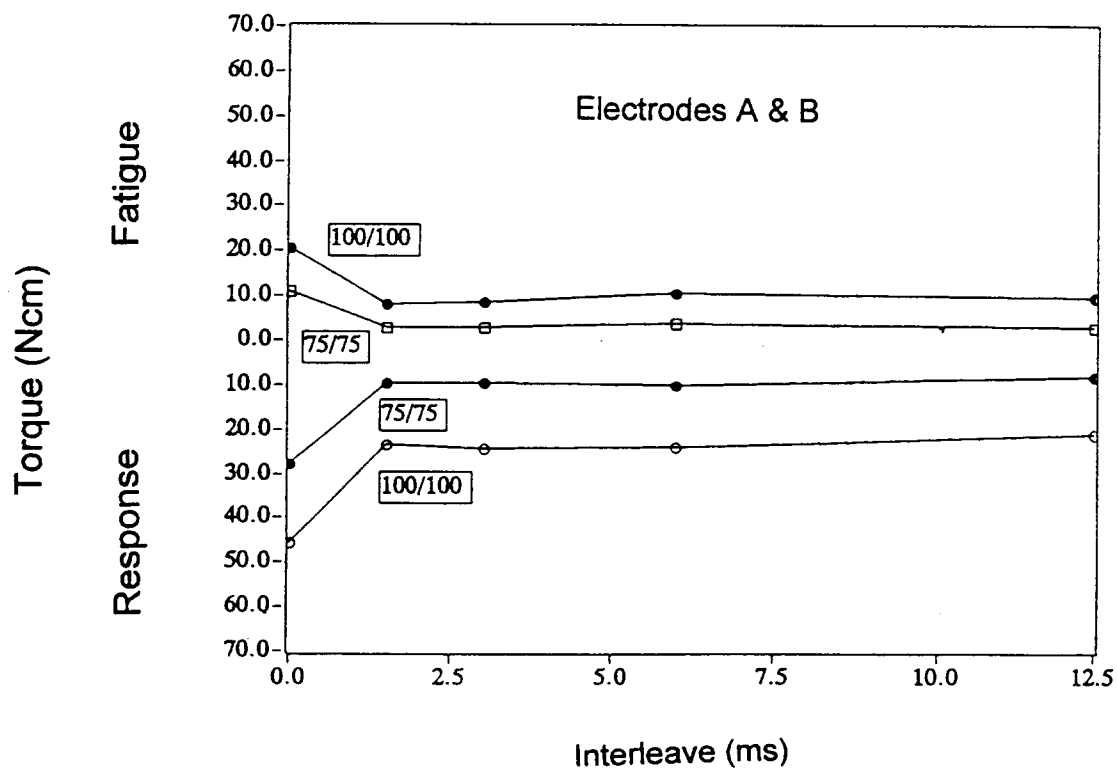


Figure 6

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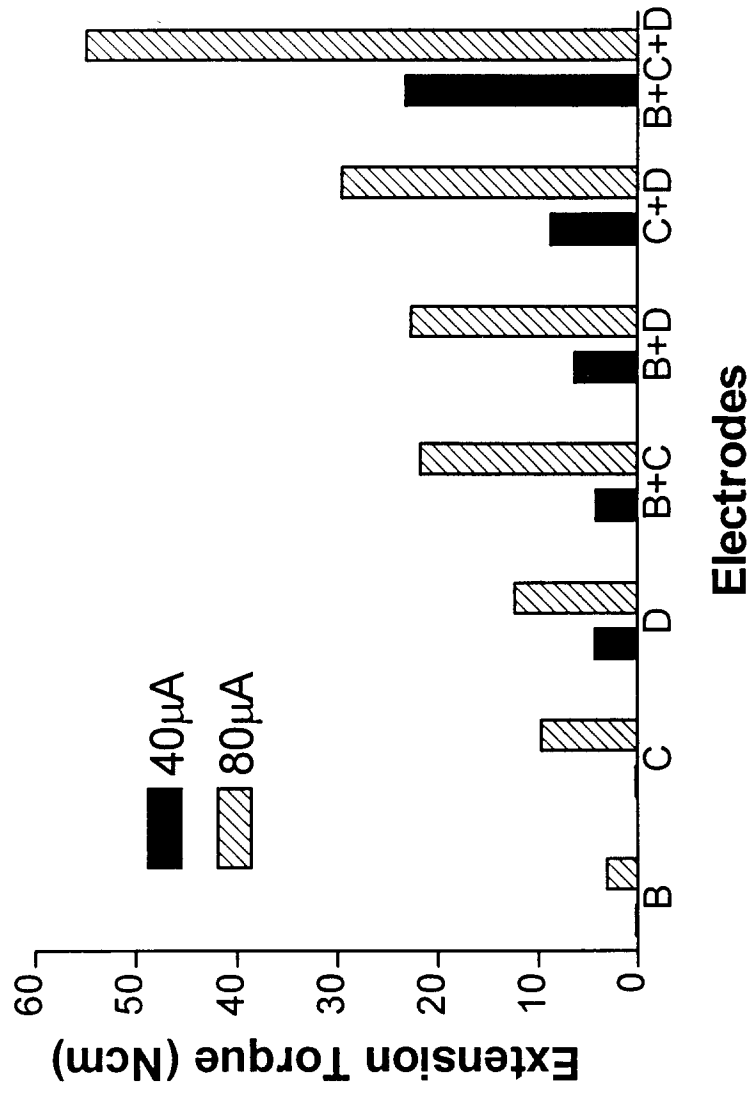


Figure 7